



Reagents and supplies for Zika IgM ELISA

Coating buffer: Carbonate/bicarbonate buffer pH 9.6

1.59g Na₂CO₃ + 2.93g NaHCO₃ diluted in 1L water.

Wash buffer: Phosphate buffered saline (PBS), 0.05% Tween 20, pH 7.2. PBS is available in powdered form from multiple commercial sources.

Blocking buffer: PBS/5% milk/ 0.5% Tween 20

Coating antibody: Goat anti-human IgM

Kirkegaard and Perry Laboratories cat# 01-10-03

Positive control: 6B6C-1 huIgM chimera (CDC) or Zika IgM-positive serum.

Negative control: known virus-negative serum

Viral antigen: Sucrose-acetone extracted suckling mouse brain viral antigens or gamma-irradiated tissue culture antigen, non-infectious, previously titrated (CDC)

Normal antigen: Sucrose-acetone extracted suckling mouse brain antigen or tissue culture antigen from mock-infected animals (CDC)

Detecting antibody conjugate: Horseradish peroxidase conjugated monoclonal antibody 6B6C-1 (commercially available from Hennessy Research and InBios).

Substrate: 3,3',5, 5' tetramethylbenzidine base (Enhanced K-Blue TMB substrate), Neogen Corp. Cat# 308175

Stop solution: TMB Stop Solution KPL Cat# 50-85-05 (75 µl/well) or 1 N H₂SO₄ (50 µl/well) (EUA)

Plates: Immulon II HB flat-bottomed 96 well plates

Dynatech Technologies cat# 3455

Microplate washer

Microplate reader with 450 nm filter

Refrigerator

Incubator (37C)

Single and multi-channel pipettors

Reagent reservoirs

Ziploc bags, paper towels